

IN THE CLAIMS

The claims are as follows:

1. (Previously Presented) A composition comprising at least two recombinant adeno-associated viruses (AAV), comprising:

a) a first recombinant AAV comprising a first recombinant DNA molecule comprising linked:

- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
 - ii) a second DNA segment which comprises a *cis*-acting heterologous transcriptional regulatory element; and
 - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV;
- and

b) a second recombinant AAV comprising a second recombinant DNA molecule comprising linked:

- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
- ii) a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and
- iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV,

wherein the recombinant DNA molecules of the two rAAVs, when contacted with a host cell, become linked, forming a molecule which has the *cis*-acting heterologous transcriptional regulatory element 5' to the open reading frame, wherein the *cis*-acting heterologous transcriptional regulatory element is positioned in the first recombinant DNA molecule so that after linking the *cis*-acting heterologous transcriptional element regulates transcription of the gene product encoded by the open reading frame, wherein if the *cis*-acting heterologous transcriptional regulatory element is an enhancer, transcription of the open reading frame is enhanced by the enhancer, wherein if the *cis*-acting heterologous transcriptional regulatory element is a promoter, transcription of the open reading frame is initiated at the promoter, and wherein the first recombinant DNA molecule does not encode a protein.

2. (Withdrawn) The composition of claim 1 further comprising a delivery vehicle.
3. (Withdrawn) The composition of claim 2 where the vehicle is a pharmaceutically acceptable carrier.
- 4-7. (Canceled)
8. (Withdrawn) The composition of claim 1 wherein the second DNA segment of the first recombinant DNA molecule comprises an enhancer.
9. (Previously Presented) The composition of claim 1 wherein the second DNA segment of the first recombinant DNA molecule comprises a heterologous promoter.
10. (Withdrawn) The composition of claim 1 wherein the second DNA segment of the second recombinant DNA molecule comprises the open reading frame but not a heterologous promoter.
11. (Withdrawn) The composition of claim 10 wherein the second DNA segment of the first recombinant DNA molecule comprises a heterologous promoter.
- 12-18. (Canceled)
19. (Previously Presented) A first recombinant adeno-associated viral vector comprising at least one *cis*-acting heterologous transcriptional regulatory element functional in a host cell, which *cis*-acting heterologous transcriptional regulatory element is positioned in the vector so that the *cis*-acting heterologous transcriptional element is capable of regulating, in the host cell, transcription of an entire open reading frame for a therapeutic gene product encoded by a second recombinant adeno-associated viral vector, after sequences in the first and second recombinant adeno-associated virus vectors become linked in the host cell, wherein the *cis*-acting heterologous transcriptional regulatory element is a promoter; and transcription of the open

reading frame is initiated at the promoter, and wherein the first recombinant adeno-associated viral vector does not encode a protein.

20. (Canceled)

21. (Withdrawn) The vector of claim 19 wherein the element is an enhancer.

22. (Canceled)

23. (Withdrawn) A plasmid comprising the vector of claim 19.

24. (Canceled)

25. (Previously Presented) A host cell contacted with at least two recombinant AAV, wherein a first recombinant AAV (rAAV) comprises a first recombinant DNA molecule comprising linked:

- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
 - ii) a second DNA segment which comprises a promoter; and
 - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV;
- and

wherein a second rAAV comprises a second recombinant DNA molecule comprising linked:

- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
- ii) a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and
- iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV, wherein the promoter in the first rAAV regulates transcriptional expression of the gene product encoded by the open reading frame in the second rAAV in a host cell contacted with the first and second rAAVs, and wherein the first rAAV does not encode a protein.

26. (Previously Presented) A method to transfer recombinant DNAs to a host cell, comprising: contacting the host cell with at least two rAAV, wherein a first rAAV comprises a first recombinant DNA molecule comprising linked:
 - i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
 - ii) a second DNA segment which comprises a promoter; and
 - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV;and wherein a second rAAV comprises a second recombinant DNA molecule comprising linked:
 - i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
 - ii) a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and
 - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV, wherein the second rAAV does not comprise a heterologous promoter 5' to the open reading frame, and wherein the first rAAV does not encode a protein.
27. (Withdrawn) A method to transfer and express a polypeptide in a host cell comprising contacting the host cell with the composition of claim 1.
28. (Withdrawn) The method of claim 26 or 27 wherein the second DNA segment of the first recombinant DNA molecule comprises a portion of an open reading frame operably linked to a promoter.
29. (Withdrawn) The method of claim 28 wherein the first recombinant DNA molecule comprises a splice donor site 3' to the open reading frame.
30. (Withdrawn) The method of claim 29 wherein the second DNA segment of the second recombinant DNA molecule comprises the remainder of the open reading frame which together

with the second DNA segment of the first recombinant DNA molecule encodes a full-length polypeptide.

31. (Withdrawn) The method of claim 30 wherein the second DNA segment of the second recombinant DNA molecule comprises a splice acceptor site 5' to the remainder of the open reading frame.

32. (Withdrawn) The method of claim 26 or 27 wherein the second DNA segment of the first recombinant DNA molecule comprises an enhancer.

33. (Withdrawn) The method of claim 26 or 27 wherein the second DNA segment of the first recombinant DNA molecule comprises a promoter.

34. (Withdrawn) The method of claim 32 wherein the second DNA segment of the second recombinant DNA molecule comprises at least a portion of an open reading frame.

35. (Withdrawn) The method of claim 33 wherein the second DNA segment of the second recombinant DNA molecule comprises at least a portion of an open reading frame.

36. (Withdrawn) The method of claim 34 wherein the second DNA segment of the second recombinant DNA molecule further comprises a promoter operably linked to the open reading frame.

37. (Withdrawn) The method of claim 35 wherein the second DNA segment of the second recombinant DNA molecule further comprises a promoter operably linked to the open reading frame.

38-45. (Canceled)

46. (Previously Presented) The composition of claim 1 wherein the second DNA segment of one of the vectors comprises a heterologous transcriptional regulatory element.
47. (Canceled)
48. (Withdrawn) The method of claim 26 or 27 wherein the second DNA segment of one of the vectors comprises a heterologous transcriptional regulatory element.
49. (Withdrawn) A method to enhance the expression of a polynucleotide in a host cell, comprising: contacting a host cell comprising a recombinant AAV vector comprising a polynucleotide segment which encodes a polypeptide, with a composition comprising a further recombinant AAV vector corresponding to the vector of claim 19 in an amount which enhances expression of the polynucleotide.
50. (Withdrawn) A method to enhance the expression of a polynucleotide in a host cell, comprising: contacting a host cell comprising a recombinant AAV vector corresponding to the vector of claim 19, with a composition comprising a further recombinant AAV vector comprising a polynucleotide segment which encodes a polypeptide, in an amount which enhances expression of the polynucleotide.
51. (Withdrawn) A method to enhance the expression of a polynucleotide in a host cell, comprising: contacting a host cell with a recombinant AAV vector corresponding to the vector of claim 19 and a further recombinant AAV vector comprising a polynucleotide segment which encodes a polypeptide, in an amount which enhances expression of the polynucleotide in the cell.
52. (Withdrawn) The method of claim 49 or 50 wherein the composition further comprises a delivery vehicle.
53. (Withdrawn) The method of claim 52 wherein the delivery vehicle is a pharmaceutically acceptable carrier.

54. (Withdrawn) The method of claim 49, 50 or 51 wherein heterologous transcriptional regulatory element in the recombinant AAV corresponding to the vector of claim 19 is a promoter.

55-57. (Canceled)

58. (Previously Presented) The vector of claim 19 wherein expression of the gene product in the host cell does not rely on splicing.

59. (Previously Presented) The composition of claim 1 wherein expression of the gene product in the host cell does not rely on splicing.

60. (Previously Presented) A first rAAV comprising a first recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises a promoter; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV, wherein the first rAAV does not encode a protein; and

a second rAAV comprising a second recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV,

wherein the promoter in the first rAAV regulates transcriptional expression of the gene product encoded by the open reading frame in the second rAAV in a host cell contacted with the first and second rAAVs.

61. (Previously Presented) A first rAAV comprising a first recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises a promoter; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV, wherein the first rAAV does not encode a protein; and

a second rAAV comprising a second recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV,

wherein the second rAAV does not comprise a heterologous promoter 5' to the open reading frame.

62. (Previously Presented) The first rAAV of claim 60 wherein the second DNA segment of the first rAAV further comprises an enhancer.

63. (Previously Presented) A composition comprising a first rAAV comprising a first recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises a promoter; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV, wherein the first rAAV does not encode a protein; and

a second rAAV comprising a second recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV,

wherein the promoter in the first rAAV regulates transcriptional expression of the gene product encoded by the open reading frame in the second rAAV in a host cell contacted with the first and second rAAVs.

64. (Previously Presented) The composition of claim 63 further comprising a pharmaceutically acceptable carrier.

65. (Previously Presented) The first rAAV of claim 61 wherein the second DNA segment of the first rAAV further comprises an enhancer.

66. (Previously Presented) A composition comprising a first rAAV comprising a first recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises a promoter; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV, wherein the first rAAV does not encode a protein; and

a second rAAV comprising a second recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV,

wherein the second rAAV does not comprise a heterologous promoter 5' to the open reading frame.

67. (Previously Presented) The composition of claim 66 further comprising a pharmaceutically acceptable carrier.

68. (Previously Presented) A host cell contacted with at least two rAAV, wherein a first rAAV comprises a first recombinant DNA molecule comprising linked:

- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
 - ii) a second DNA segment which comprises a promoter; and
 - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV;
- and

wherein a second rAAV comprises a second recombinant DNA molecule comprising linked:

- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
- ii) a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and
- iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV, wherein the second rAAV does not comprise a heterologous promoter 5' to the open reading frame, and wherein the first rAAV does not encode a protein.

69. (Previously Presented) A method to transfer recombinant DNAs to a host cell, comprising: contacting the host cell with at least two rAAV,
- wherein a first rAAV comprises a first recombinant DNA molecule comprising linked:
- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
 - ii) a second DNA segment which comprises a promoter; and
 - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV;
- and
- wherein a second rAAV comprises a second recombinant DNA molecule comprising linked:
- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
 - ii) a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and
 - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV,
- wherein the promoter in the first rAAV regulates transcriptional expression of the gene product encoded by the open reading frame in the second rAAV in a host cell contacted with the first and second rAVVs, and wherein the first rAAV does not encode a protein.